7-1963

An Investigation of the Influence of the Suprapharyngeal Ganglion upon Oxygen Consumption of Resting Lumbrious Terrestris

William E. Elzinga

AN INVESTIGATION OF THE INFLUENCE OF THE SUPRAPHARYNGEAL GANGLION UPON OXYGEN CONSUMPTION OF RESTING LUMBRICUS TERRESTRIS

by
William E. Elzinga

A thesis presented to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
July 1963

ACKNOWLEDGEMENTS

The investigator wishes to express his sincere appreciation to the many persons for their assistance in obtaining materials and providing advice for the production of this paper.

Special mention is due to the members of my graduate committee, Dr. Jean McVay Lawrence, Dr. Thane S. Robinson, and Dr. Imy V. Holt. The author also wishes to thank Dr. Leo C. VanderBeek for the use of his laboratory.

William E. Elzinga

TABLE OF CONTENTS

PAGE

ACKNOWLEDGEMENTS.....1

INTRODUCTION.....1

METHODS.....4

RESULTS.....11

DISCUSSION.....14

SUMMARY.....18

LITERATURE CITED.....19

INTRODUCTION

Because cephalization and centralization of the earthworm are more pronounced than in less complex forms, it is a relatively simple operation to remove a portion of the central nervous system to investigate its functions. However, the extirpation of various parts of the central nervous system of the earthworm has been the subject of only a few research papers. It is the purpose of this study to investigate the influence of extirpation of the suprapharyngeal ganglia on the oxygen uptake of the resting earthworm, Lumbricus terrestris.

Of the studies concerned with the influence of extirpation of some parts of the central nervous system of Annelids on gaseous exchange and other physiological functions, by far the greatest interest has been centered on the suprapharyngeal ganglia.

Bennett and Suttle (1960) removed the suprapharyngeal ganglion from the earthworm, L. terrestris, and found that it is the possible source of factors which affect general metabolism and seems to have an inhibitory effect on the seminal vesicles and

receptacles of the earthworm. Clark and Clark (1959) found that the removal of the suprapharyngeal ganglion at the same time as that of a number of posterior body segments inhibits posterior regeneration. The effect of the removal of the suprapharyngeal ganglia from an earthworm is described in Prosser and Brown (1962). The anterior segments are lifted, the worm crawls normally, appears restless and active, can right itself, copulates, eats and burrows in one half hour as compared with the normal time of one to two minutes. Kovaleva (1961) found that the removal of the suprapharyngeal ganglion led to intensification of the animal's motor activity. Further, the oxygen consumption of the worm at rest was increased and its body weight diminished gradually.

It has been established that neurosecretory cells are present in the suprapharyngeal ganglion of the earthworm. They have been thoroughly studied and described by Scharrer and Brown, (1961). The function of these granules has not been established. However, it is assumed that they may be hormonal in nature (Scharrer and Brown, 1962).

According to Kovaleva (1961) the motor activity of the earthworm is intensified with the removal of

The suprapharyngeal ganglion. This increase in motor activity in turn increases the oxygen consumption of the worm and tends to mask any influence that the suprapharyngeal ganglion may have on tissue metabolism. In the present study attempts were made to control the activities of the worms by lowering the ambient temperature and reducing the environmental light to nearly darkness in order to measure oxygen consumption of the worms at rest. In this way, the results should indicate if the suprapharyngeal ganglion contains a factor or factors which influence the rate of oxygen consumption rather than the reflection of increased motor activity.

METHODS

The earthworms used in this study were purchased from Walt's Bait House, Kalamazoo, Michigan. The worms were not cultured by the dealer, but were purchased from collectors who gathered them from the local area. The worms were identified by the investigator as Lumbricus terrestris using Eddy and Hodson (1961:30-1).

For the experimental group 320 worms, all having mature clitella, were used. They were randomly separated into lots of tens and placed in plastic containers filled with fresh Sphagnum. The time of extirpation of the suprapharyngeal ganglion was recorded for each group of ten in order that the mean oxygen consumption of the ten worms could be followed for a period of days.

The control group, all having mature clitella, were separated into lots of fifty and placed in plastic containers (2000 ml. capacity), and then filled with Sphagnum. Both of the groups were then placed in a refrigerator in which the temperature was 12°C. and left there for a period of seven days.

After the seven day acclimation period, the extirpation of the suprapharyngeal ganglion was carried out on the 320 experimental worms using the following procedure:

1. A group of ten worms was placed in tap water and kept in a freezer for fifteen minutes. This reduced the activity of the worms.
2. To expose the suprapharyngeal ganglia, an incision was made along the dorsal surface of the second through the fifth segment.
3. Although the suprapharyngeal ganglia were seldom exposed after the incision, by using a curved tip finder, they could be lifted from between the third and fourth segments and quickly excised with small sharp scissors.
4. The ten worms were then replaced in the Sphagnum and refrigerated. Three days were required to extirpate the suprapharyngeal ganglia of the 320 experimental worms.

A Wärburg respirometer was used to measure the oxygen consumption of the worms. Mercury was used to measure and calibrate the volume of the flasks and manometers of the Wärburg. Brodies solution (Umbreit, et al., 1957), with a density of 1.033, was used as the manometric fluid.

Since the Wärburg respirometer had no refrigerator unit, it was necessary to devise a method to maintain and control an appropriate temperature. The addition of ice to the water bath was not satisfactory because the temperature varied 1 to 2°C. The manometers used were extremely sensitive to a very slight temperature change (± 0.5°C.). It was found however, that by transporting tap water into the bottom of the water bath and siphoning it from the top that a reasonably constant temperature (12.5 ± 0.5°C.) could be maintained. Since L. terrestris is relatively inactive at 13°C. and below, the temperature of 12.5°C. was used for measurements of oxygen uptake of the resting worms, (Grant, 1953, Wolf, 1938, and Roots, 1956).

The procedure used in preparing the flasks for the worms was as follows:

1. Ten reaction flasks and manometers were used for the experimental group, four for the control and two were operated as thermobarometers to measure temperature and barometric changes.
2. In the bottom of each reaction flask a piece of filter paper saturated with water was inserted to prevent the worms from dehydrating.
3. The worms were then placed in the flasks after they had been washed with (12.5°C.) tap water to remove surface dirt.

4. The flasks were weighed collectively before and after the worms were placed in them to obtain the combined wet weight of the ten worms.

5. Thirty per cent aqueous solution of potassium hydroxide was placed in the carbon dioxide absorption cell.

6. The flasks and the worms were then placed in the Wärburg water bath with the stopcocks of the manometers open for one half hour at 12.5°C.

7. After this equilibrium period the stopcocks were closed and the pressure changes recorded every ten minutes for one hour. These readings were taken every ten minutes because they were found to be more accurate and convenient than separate one hour readings.

8. After the reaction flasks containing the worms were placed in the Wärburg water bath for the one half hour acclimation period, and before the pressure changes were recorded, the manometric fluid of the closed arm of the U-tube was adjusted to the 150 mm. mark.

9. When the system was closed and the worms began to consume the oxygen, a decrease in pressure within the reaction flask was observed (the fluid in the closed arm began to rise).

When the fluid of the closed arm was adjusted back to the 150 mm. mark, the change of pressure was recorded from the difference between the open and closed arms.

Since the open arm of the manometer was exposed to atmospheric pressure changes and the flasks to temperature flucuations, two thermobarometers were used to correct these variable factors. If the barometric pressure varied throughout the experiment, it was recorded on the thermobarometer as well as any temperature variations. These changes were then compensated for in calculating the final pressure change.

When the pressure changes for each reaction flask had been recorded in millimeters for the entire hour interval, and all the necessary calibrations made from thermobarometers, they were then multiplied by a constant (k) to obtain the microliters of oxygen consumed per worm per hour. This computation was necessary in order to reduce the results, which were recorded in millimeters to microliters consumed at standard temperature and pressure (S.T.P.). This constant (k) was calculated using the following formula (Umbreit, et al., 1957):

$$k = \frac{V_g \frac{273}{T} + V_f}{P_o}$$

V_g = Total mean volume of the flasks (as calculated from the original calibration of the flasks) minus mean volume of worms as calculated from total number of worms used. (20,922 ul.-4,500 ul.).

T = Absolute temperature plus temperature of bath (-273°C. + 12.5°C.).

V_f = Fluid volume (mean volume of all worms) (4,500 ul.).

S_1 = Solubility of oxygen in water (nil).

P_o = Pressure of Brodie's solution at S.T.P. (10,000 mm. of mercury).

k = 1.59 (The constant derived from the calculations)

After the oxygen uptake of each set of ten worms was recorded, the mean oxygen uptake for one worm was calculated from a group of twenty worms. Also, the mean weight of the worms was calculated for one worm from a group of twenty worms. From these calculations, since the weight variation of the worms was small ($\pm$ 1 gram), a perfect positive correlation coefficient, between weight and oxygen consumption, was used to present the oxygen uptake as milliliters of oxygen consumption per gram of wet weight of worm per hour (ml. O_2/gram wt./hr).

Other investigators (Saroja 1959, Whitney 1942, and Zeuther 1953) have found that the correlation coefficient between oxygen consumption and weight was 0.75. However, this correlation coefficient was figured for large variations in weight.

RESULTS

The effect of the removal of the supra-pharyngeal ganglion from the earthworm, Lumbricus terrestris, was to depress the oxygen consumption of the resting worms, as compared with the normal oxygen consumption of the resting control worms. This depression was most evident throughout the first four days after the operation (Figure I). The

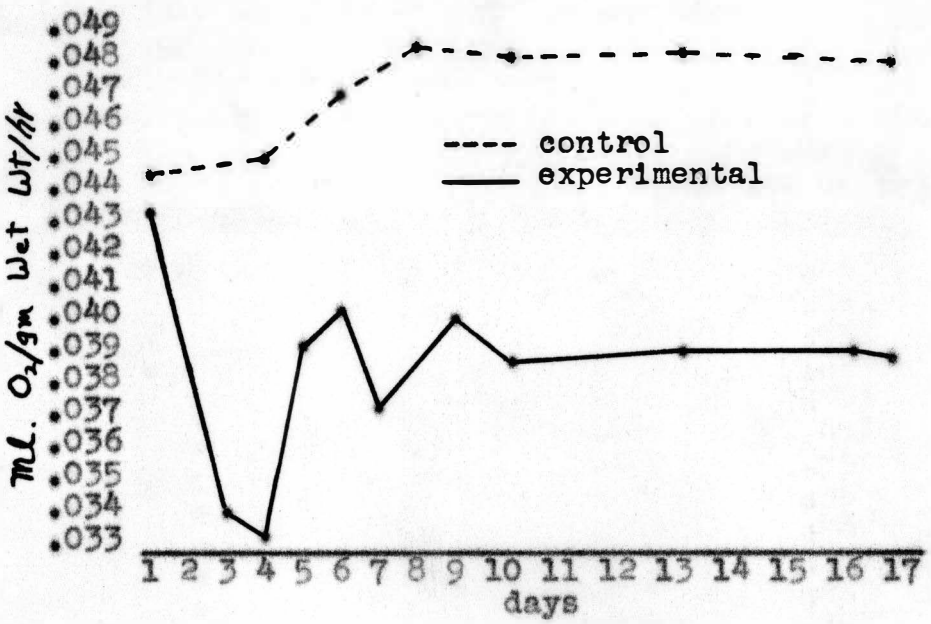

Figure I. A comparison of the rates of oxygen consumption of the experimental and control worms.

following three days, fourth through sixth day, the oxygen uptake of the experimental worms increased at about the same rate that it decreased the first three

days. However, when the oxygen consumption of the experimental worms reached a plateau, the sixth day, it was lower than that of the control group.

The mean oxygen uptake for each day after the operation (Table I) shows that the experimental worms consumed on the average less oxygen than the normal worms at all times with the exception to the first day.

TABLE I.
A summary of the mean oxygen consumption, at 12.5°C., for the experimental and control groups.

Days	No. of worms		Oxygen consumption (ml. O_2/gm wet wt/hr)	
	Experimental	Control	Experimental	Control
1	20	8	.044	.043
2	"	"	.040	.041
3	"	"	.034	.051
4	"	"	.033	.044
5	"	"	.040	.056
6	"	"	.040	.050
7	"	"	.039	.050
8	"	"	.037	.047
9	"	"	.043	.047
10	"	"	.039	.050
11	"	"	.034	.053
12	"	"	.038	.047
13	"	"	.040	.042
14	"	"	.041	.047
15	"	"	.038	.054
16	"	"	.041	.052
	Grand mean =		.039	.045

When the percentage of difference between the oxygen uptake of the experimental and control groups

was measured, the difference was found to be small (approximately 3 per cent) for the first twenty four hours. This difference increased rapidly from the second through the fourth day. The fifth day, the percentage of difference decreased slightly and leveled off at approximately nine per cent (Figure II).

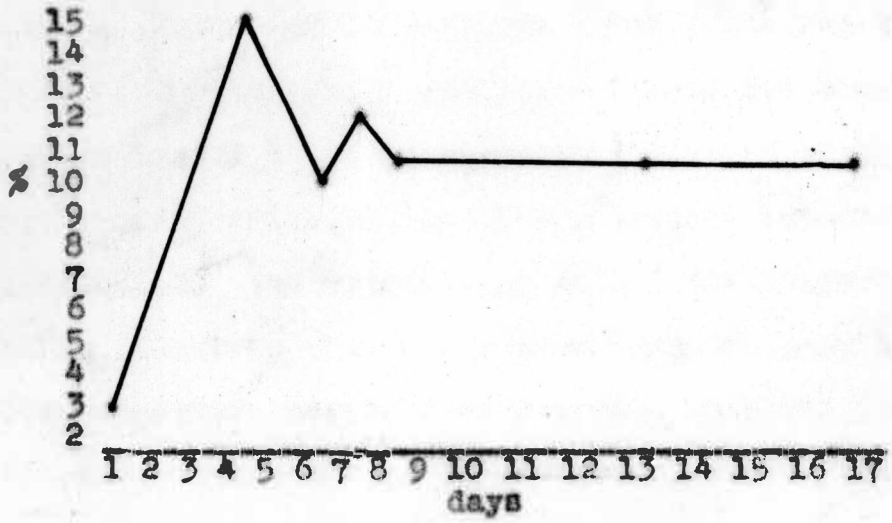

Figure II. The percentage of difference between the oxygen consumption of the experimental and control groups.

DISCUSSION

In removing the suprapharyngeal ganglion, most of the connective nerves, those leading to the prostomium and parts of the circumpharyngeal connectives, were also removed unless care was taken to leave them intact. According to Kovaleva (1961), the removal of the suprapharyngeal ganglion without the connective nerves leads to an intensification of the worm's motor activity and thus increases its oxygen consumption. The oxygen consumption was measured at 3-4°C. However, when the suprapharyngeal ganglion plus connective nerves were removed, Kovaleva found that oxygen consumption dropped briefly but later rose again.

The second results agree with the findings of the present study, carried out at 12.5 ± 0.5°C. When the suprapharyngeal ganglion was removed, no effort was made to leave the connecting nerves intact. In Kovaleva's (1961) study, no mention was made of the leveling-off of the oxygen consumption of the worms and whether or not it remained lower than the controls as it did in the present study.

From the literature it appears that there are two functions of the suprapharyngeal ganglion. The first, and that which has been investigated most thoroughly, is its sensory nature. Sensory receptors, such as tactile, chemical and light, are scattered throughout the epidermis of the earthworm, but are most concentrated in the prostomium and anterior segments (Prosser and Brown, 1962). The second major function of the suprapharyngeal ganglion, one which is apparently neuroendocrine in nature, has been shown best by Clark and Clark (1959). They have shown, in posterior regeneration of the earthworm, that removal of the suprapharyngeal ganglia, at the same time as a number of posterior segments, inhibits posterior regeneration. However, if the ganglia are removed 24-48 hours after the posterior segments are amputated, posterior regeneration proceeds normally. Furthermore, in making tissue sections of the suprapharyngeal ganglia six hours after amputation of the posterior segments, the neurosecretory cells of these ganglia were demonstrated to have increased their secretion. These secreting substances may be carried via the circulatory system. Therefore, it is evident that there are substances secreted by the suprapharyngeal ganglia of L. terrestris.

Those investigators who have removed the supra-pharyngeal ganglia found that the following additional effects do exist: a loss of body weight, inhibitory effects on the seminal vesicles and receptacles, factors which affect regeneration and variations in tissue metabolism, Bennett and Suttle (1960), Clark and Clark (1959), Kovaleva (1961), O'Brien (1957) and Prosser and Brown (1962).

In studying the relationship of the supra-pharyngeal ganglion and the subpharyngeal ganglion, it was found that the former has an inhibitory or restraining control over the motor center in the latter (Kovaleva, 1961). Since the suprapharyngeal ganglia apparently inhibit motor activity, the absence of the ganglion should and does increase the activity of the worm which in turn would increase its oxygen consumption. This in part, may explain why the tissue metabolism of the earthworm varies at normal temperature. However, when the temperature is reduced as in Kovaleva's (1961) and the present study, so that the motor activity is depressed to resting conditions, the oxygen consumption of the worm is not positively influenced by the motor center. Yet the oxygen consumption, as the results of the present study indicate, does vary from that of the control worms.

Since the leveling off of the resting oxygen consumption was found to be lower than the controls, it is very likely that the suprapharyngeal ganglia contains factors which regulate oxygen uptake of the resting worm.

SUMMARY

The purpose of the present study was to investigate the influence of extirpation of the suprapharyngeal ganglion on the oxygen consumption of the resting earthworm, L. terrestris.

To obtain pertinent data, the suprapharyngeal ganglion was removed from 320 experimental worms. The oxygen consumption of these worms, as well as that of the control worms, was observed at 12.5 ± 0.5°C., using a Warburg respirometer.

The results of the study show that the oxygen consumption of the experimental worms at rest decreased as compared with the oxygen uptake of the controls. The oxygen consumption fell sharply for the first four days after the operation and then increased just as sharply until the sixth day. It then tended to level off at about ten hundreths of a milliliter lower than than of the controls.

Since the oxygen consumption was not due to increased motor activity, it was concluded that the suprapharyngeal ganglion contains factors which influence gaseous exchange in the resting state.

LITERATURE CITED

Bennett, M.R. and Suttle, G.E., 1960. "Effects of the Removal of the Suprapharyngeal Ganglion in Earthworms." Anat. Rec., 137(3):339.

Clark, R.B. and Clark, M.E., 1959. "Role of the Suprapharyngeal Ganglion during the Early stages of Caudal Regeneration in some Errant Polychaetes." Nature, 183(4678): 1834-5.

Eddy, S. and Hodson, A.C., 1961. Taxonomic Keys. 3rd ed. Minneapolis:Burgess Co. Pp. v + 162.

Grant, W.E., 1953. "Temperature Tolerance in Earthworms." Anat. Rec., 117:561.

Kovaleva, N.E., 1961. "The Effects of Destruction of Parts of the Central Nervous System on Gas Exchange and other Physiological Functions in the Earthworm." Fiziol. Zhur. SSSR. (transl.) 47(1):114-20.

O'Brien, R.A., 1957. "Tissue Metabolism During Posterior Regeneration in the Earthworm." Aust. Jour. Exp. Biol. and Med. Sci., 35(4): 373-80.

Prosser, C.L. and Brown, R.A., 1962. Comparative Animal Physiology. Philadelphia: W. B. Saunders Co. 688. Chapter 21, pp.624-5.

Roots, B.I., 1956. "The water Relations of Earthworms." Jour. of Exp. Biol., 33:29-44.

Saroja, K., 1959. "Studies on the O_2 consumption in Tropical Poikilotherms. II. Oxygen Consumption in Relation to Body Size and Temperature in the Earthworm, Megascolex mauritti, when kept Submerged under Water.".Proc. Ind. Acad. Sci., Sect. B., 49(3): 183-93.

Scharrer, E. and Brown, S., 1961. "Neurosecretion. XII. The Formation of Neurosecretory Granules in the Earthworm, Lumbricus terrestris." Zeit. Zellf. 54:531-40.

_____, and _____, 1962. "Neurosecretion in Lumbricus terrestris." General Comparative Endocrinology. 2:1-4.

Umbreit, W.W., Burris, R.H. and Stauffer, J.F., 1957. Manometric Techniques. Minneapolis:Burgess Co. Pp. iii + 338., 1-6 and 66.

Whitney, R.J., 1942. "The Relationship of Animal Size to Oxygen Consumption in some Freshwater Turbellarian Worms." Jour. of Exp. Biol., 19:233-42.

Wolf, A.V., 1938. "Studies on the Behavior of Lumbricus terrestris to Dehydration, and Evidence for a Dehydration Tropism. Ecol., 19:346-8.

Zeuther, E., 1953. "Oxygen Uptake as Related to Body Size in Organisms." Quart. Rev. Biol. 28: 1-12.